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Worldwide Regulatory Affairs

Wyeth

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May 18, 2005

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Re: Docket No. 2005D-0047: February 18, 2005 (70 FR 8378-8379)

Dear Sir/Madam:

Wyeth Pharmaceuticals is submitting the following comments on the *Draft Guidance for Industry: Considerations for Plasmid DNA Vaccines for Infectious Disease Indications* (dated February 2005).

Wyeth appreciates the opportunity to comment on the Plasmid DNA Guideline and trusts that the Agency will take these comments into consideration when preparing the final guidance document. We recognize that the evaluation of the potential risks associated with the clinical use of DNA vaccines must be thorough and done with great care. This approach is in alignment with Wyeth's commitment to produce vaccines that are both safe and efficacious. We have been aided in working towards that goal by the flexibility and openness to discussion the Agency has shown over time as new situations arise in our DNA plasmid vaccine development. This Guidance will assist in future development in that it reflects the knowledge and experience gained on DNA plasmids since the last guidance was published.

In our attached comments we indicate sections where we believe further clarification would be helpful. While this document provides a useful guideline for vaccine evaluation, because there are continually new issues presented by evolving technologies, we believe it is best to hold to the default that each new vaccine must ultimately be assessed on a case-by-case basis.

Sincerely,

Susan J. Sciotto-Brown
Senior Manager, Worldwide Regulatory Affairs

Jack B. Love
Assistant Vice President, Worldwide Regulatory Affairs

2005D-0047

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Guidance for Industry
Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

Comments from Wyeth Vaccines, May 2005
Excerpts from the Guidance are in **bold**.

I. Introduction

No comment.

II. Manufacturing Issues

A. Product Manufacture

"We recommend that you test both the MCBs and WCBs to ensure that they are free from bacteriophage and other adventitious agent contamination and that you establish the genetic stability of the MCB and WCB." (p. 3, paragraph 2)

We believe that it is most important to test the Research Cell Bank (RCB) to ensure that it is free of contaminants that could be carried through the process. If the Master Cell Bank (MCB) and Working Cell Bank (WCB) are made in the same facility, then it is very unlikely that these contaminants would be introduced during the preparation of the WCB. Therefore, we propose that bacteriophage and adventitious agents testing are performed on the RCB and MCB only and not be required for the WCB when it is made in the same facility as the MCB.

Please clarify that it is the stability of the plasmid that is being requested and not stability of the *E. coli* carrying the plasmid.

B. Bulk Plasmid Product Release Testing

"We recommend that you evaluate assay methods by testing known amounts of reference materials or spiked samples, or by other appropriate measures, and that you submit to CBER data documenting assay performance." (p. 3, paragraph 4)

A lot of data is generated in the qualification, and later, validation, of the assays used in product release testing. Please clarify how much information on these assays CBER would like included in the IND. We suggest that a summary of assay performance might provide CBER sufficient information to assure the assays are performing adequately, especially in the early clinical phases.

"We recommend that as product development proceeds towards licensure, you provide evidence that *in vitro* potency correlates with *in vivo* immunogenicity." (p. 4, paragraph 3)

In the early stages of vaccine development, the vaccine is often made of multiple components, each requiring separate release testing. We therefore recommend that the draft guidance clarify that this request for immunogenicity applies to the final formulated vaccine, since demonstrating *in vitro* or *in vivo* immunogenicity of the individual components would likely be irrelevant.

We agree that it is important to assess immunogenicity and establish some correlation with *in vitro* potency during preclinical development. As a release test, it is feasible to do a bioassay that quantitates plasmid expression, however, conducting an *in vivo* potency assay to measure biological activity is very time consuming, therefore such an animal study would not be practical as a release test. It is possible that a cellular assay could be developed, however, molecules designed to stimulate a cellular response may not generate sufficient antibody to be relevant and cellular assays in some common species used for toxicology studies are not possible due to a lack of suitable reagents.

III. DNA Vaccine Modifications

A. Changes to the Insert or Vector

"Changes to the DNA sequence of the insert gene or vector sequences of a DNA vaccine would require the submission of a new IND..."

No comment.

B. DNA Sequence Analysis

"We advise you to establish the identity and amount of each plasmid component in the vaccine preparation to ensure lot-to-lot consistency... there may be instances when technical limitations prevent complete sequence information from being obtained on a heterogeneous mixture of plasmids..." (p. 5, paragraph 4)

In a situation where multiple plasmids of similar size are included in the final vaccine, we would suggest acceptance of sequencing of the plasmids in the stage prior to formulation and confirmation of their presence by an identity test such as *in vitro* gene expression.

IV. Preclinical Immunogenicity and Safety

A. General Considerations

No comment.

B. Immunogenicity

"We recommend that you develop assays to assess immunological potency in animal models. This could include the evaluation of antigen-specific antibody titers, seroconversion rates, activation of

cytokine secreting cells, and/or measures of cell-mediated immune responses. Optimally, these studies are designed to collect information regarding the duration of the immune response." (p. 6)

Please clarify that the assays to assess immunological potency in animal models are intended for use during product development and non-GLP characterization studies and, as such, are not subject to Good Laboratory Practice (GLP) requirements (21 CFR, Part 58).

C. Autoimmunity

"In cases of immunity developing against a transgene product (such as a cytokine), we recommend that you examine potential cross-reactivity with the corresponding endogenous protein." (p. 7)

Please clarify the type of study that is being requested. Are such studies required if plasmid expression is shown to be short-lived or expression is below a certain threshold?

D. Tolerance

No comment.

E. Challenge/Protection, Cytokines, Prime/Boost

No comment.

F. Local Reactogenicity and Systemic Toxicity Studies

No comment.

G. Biodistribution and Integration Analysis

"We have determined that integration studies are not necessary when biodistribution/persistence studies demonstrate that plasmid DNA does not persist in any tissue of any animal at levels exceeding 30 copies per 10^5 cellular genomes at 60 days post vaccination. If the DNA plasmid persists at significantly higher copy number at any site in animal, we recommend that you study whether the DNA has integrated in the genome of the vaccinated animal." (p. 9, paragraph 2)

We believe that a cut-off of 30 copies construct/10^5 cells (i.e., ~45 copies/ug genomic DNA) is an unrealistically low number and this number may have been derived incorrectly from the references listed in the guidance (e.g., Wang, Z, et al, 2004, Gene Therapy 11, 711-721; Ledwith, BJ, et al, 2000, Intervirology 43, 258-272). Our understanding is that the limit of 30 copies construct/10^5 cells may have been chosen based on ranges that are reported for plasmid construct (and have been assumed to be essentially extra-chromosomal) after at least four successive gel separations have been conducted to separate the high molecular weight

(HMW) DNA away from remaining free construct (i.e., the Merck method for analysis of potential integration). No doubt the construct copies/ug genomic DNA analyzed directly from injection site muscle taken on a biodistribution study, i.e., prior to any gel separation steps, would be considerably higher. We suggest a more realistic acceptance level for plasmid construct copy number at injection site tissues (muscle/skin) at later time points (e.g., Day 60) on a biodistribution analysis could be hundreds to several thousand copies/ug DNA. These numbers would be expected to vary with the absolute mg amount of construct administered on Day 1, the dose volume, and or other technical factors. We agree that the construct copies/ug DNA extracted from non-injection site tissues at later time points should be <LLOQ.

Given the increasing sensitivity of the assays (qPCR) to detect potential construct integration and improvements in vaccine delivery technology (electroporation), it is likely that potential integration of plasmid constructs will be more frequently detected in the future. It would be helpful if the guidance provided clarification concerning the level of (potential) integration would be regarded as a concern. We suggest that anything below the accepted background of spontaneous gene-inactivating mutation, 10,000 copies/ug HMW DNA (see Wang, Z, et al, 2004 cited above) would be considered acceptable.